Analysis of anti-TNF-induced skin lesions reveals strong Th1 activation with some distinct immunological characteristics

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Abstract: BACKGROUND: Psoriasiform and eczematous eruptions are the most common dermatological adverse reaction linked to anti-TNF-α therapy. Yet, a detailed characterization of their immune phenotype is lacking. OBJECTIVES: We sought to characterize anti-TNF-α induced inflammatory skin lesions on a histopathologic, cellular and molecular level, compared to psoriasis, eczema (atopic dermatitis), and healthy control skin. METHODS: Histopathologic evaluation, gene expression (quantitative RT-PCR) and computer-assisted immunohistologic studies (TissueFAXS) were performed on 19 skin biopsies from IBD (n=17) and rheumatoid arthritis (n=2) patients with new-onset inflammatory skin lesions during anti-TNF-α-therapy. RESULTS: While most biopsies showed a psoriasiform and/or spongiotic (eczematous) histopathologic architecture, these lesions were inconsistent with either psoriasis or eczema on a molecular level using an established CCL27/iNOS classifier. Despite some differences in immune skewing depending on the specific histopathologic reaction pattern, all anti-TNF-α-induced lesions showed strong IFN-ɣ activation, at higher levels than in psoriasis or eczema. IFN-ɣ was most likely produced by CD3/CD4/Tbet-positive Th1 lymphocytes. CONCLUSIONS: New-onset anti-TNF-α-induced eruptions previously classified as psoriasis or spongiotic dermatitis (eczema) exhibit a molecular profile that is different from either of these disorders. This article is protected by copyright. All rights reserved.

DOI: https://doi.org/10.1111/bjd.16126

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Article type : Original Article

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RUNNING HEAD: Characterization of anti-TNF-induced skin eruptions

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/bjd.16126

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FUNDING SOURCES: This study was supported by a research grant to PMB from the Austrian Society of Dermatology and Venereology.

CONFLICT OF INTEREST DISCLOSURES: PMB received personal fees from Sanofi Genzyme and LEO Pharma. The other authors declare that there are no relevant conflicts of interest to report.

BULLET STATEMENTS

- Psoriasiform and eczematous lesions are the most common skin adverse events of anti-TNF-α treatment, yet their immune profile remains to be elucidated.
- We found that these lesions are distinct from genuine psoriasis and eczema, but are uniformly characterized by a strong Th1 inflammatory signature.

- Results might help to guide future treatment strategies for chronic inflammatory diseases preventing these skin side effects.

ABBREVIATIONS

CAMP	Cathelicidin antimicrobial peptide
DEFB4	Defensin, Beta 2
HE	Hematoxylin Eosin
HPSS	Histopathologic psoriasis severity score
IBD	Inflammatory bowel disease
NOS2	Nitric Oxide Synthase 2 (inducible Nitric Oxide Synthase/iNOS)
pDC	Plasmacytoid dendritic cell
RA	Rheumatoid arthritis

ABSTRACT

Background: Psoriasiform and eczematous eruptions are the most common dermatological adverse reaction linked to anti-TNF-α therapy. Yet, a detailed characterization of their immune phenotype is lacking.

Objectives: We sought to characterize anti-TNF-α induced inflammatory skin lesions on a histopathologic, cellular and molecular level, compared to psoriasis, eczema (atopic dermatitis), and healthy control skin.

Methods: Histopathologic evaluation, gene expression (quantitative RT-PCR) and computer-assisted immunohistologic studies (TissueFAXS) were performed on 19

skin biopsies from IBD (n=17) and rheumatoid arthritis (n=2) patients with new-onset inflammatory skin lesions during anti-TNF-α-therapy.

Results: While most biopsies showed a psoriasiform and/or spongiotic (eczematous) histopathologic architecture, these lesions were inconsistent with either psoriasis or eczema on a molecular level using an established CCL27/iNOS classifier. Despite some differences in immune skewing depending on the specific histopathologic reaction pattern, all anti-TNF-α-induced lesions showed strong IFN-γ activation, at higher levels than in psoriasis or eczema. IFN-γ was most likely produced by CD3/CD4/Tbet-positive Th1 lymphocytes.

Conclusions: New-onset anti-TNF-α-induced eruptions previously classified as psoriasis or spongiotic dermatitis (eczema) exhibit a molecular profile that is different from either of these disorders.

KEYWORDS: anti-TNF; TNF-alpha; psoriasiform; eczematous; psoriasis; eczema;

INTRODUCTION

Anti-tumor necrosis factor alpha (TNF-α) antibodies are efficacious therapeutics for chronic inflammatory diseases such as Crohn's disease, ulcerative colitis, rheumatoid arthritis (RA) and psoriasis, with an overall favorable safety profile.[1] Psoriasiform and eczematous skin eruptions have turned out to be the most frequent inflammatory skin complications of TNF-α-blocker treatment,[2-6] with a cumulative incidence of 28.9% at 10 years, leading to anti-TNF-α discontinuation in 18.6% of patients.[5] After switching to a different TNF-α-blocker, a recurrence rate of 57% was observed, suggesting a class effect.[5] While TNF-α-blockers increase blood eosinophil levels[7] and worsen pre-existing eczema,[8,9] the advent of psoriasiform

lesions came as a surprise,[10] as TNF-α-blockers are generally a well-established anti-psoriatic treatment modality.[11] Currently, psoriasiform lesions are classified as "classic" psoriasiform, inverse, palmoplantar, palmoplantar pustular, guttate, and scalp psoriasiform lesions.[12] Eczematous lesions, which are histologically characterized by a spongiotic inflammatory reaction (spongiotic dermatitis), have not been further classified.[8] Other inflammatory cutaneous side effects include lupus-like disorders, vasculitis, and granulomatous and lichenoid reactions, but these appear at much lower frequencies.[13]

A disturbed Th1/Th2 balance has been postulated as the basis for eczematous lesions,[14] suggesting TNF-α as an important Th1 component that can suppress Th2 responses,[8] with Th2 cytokines driving the formation of eczema.[15] However, deeper mechanistic insights are lacking. In the context of psoriasiform lesions, which have been shown to be rich in Th1 and Th17 immune cells and to respond to the IL-12/IL-23p40 blocker ustekinumab,[4] there is some evidence that TNF-α-blockade can lead to unopposed interferon/IFN-α production by plasmacytoid dendritic cells (pDCs),[3,10,16-18] which have also been suggested as key drivers in the development of conventional psoriasis.[19-21] However, sifalimumab, an IFN-α-blocker, failed to elicit effects on psoriasis in a clinical trial,[22] challenging the central role of IFN-α in this disease, at least in fully established lesions.

Despite the fact that several studies have described potential driver cytokines in anti-TNF-induced skin lesions,[3,4,17,18,23] a comparative, broad immunological characterization of anti-TNF-α-induced inflammatory skin lesions is still lacking. We thus decided to comprehensively assess them on a histopathologic, molecular and cellular level. We found that anti-TNF-α-induced "psoriasiform" and

"eczematous/spongiotic" skin lesions lack some key immune features of psoriasis and eczema, with strong Th1- and IFN-α-associated inflammation.

PATIENTS, MATERIALS AND METHODS

Patient characteristics and skin samples

19 patients on anti-TNF-α-treatment were referred to our outpatient clinic due to new-onset scaly erythematous skin lesions (Table 1). 17 (89.5%) were treated for IBD (15 with Crohn's disease, 2 with ulcerative colitis), and 2 for rheumatoid arthritis (RA). 14, 4 and 1 were on adalimumab, infliximab, or golimumab, respectively. Mean age was 40.4 (SD 13.9) years, and the median duration of therapy was 24 months (range: 7-105 months). 17/19 patients (89.5%) were negative for ANAs. There was neither a prior history of psoriasis nor of eczema (or associated atopy) in any individual, and no one reported on injection site reactions. Four-millimeter punch biopsies were obtained from clearly inflamed skin without prior topical treatment. Biopsies from patients with classic plaque-type psoriasis (n=6), atopic eczema (atopic dermatitis, n=9) and from healthy controls (n=5) served as comparators. Biopsies were taken under an IRB-approved protocol (Ethics Committee of the Medical University of Vienna, Austria; approval #071/2005) according to the Declaration of Helsinki. All participants gave written informed consent prior to inclusion.

Quantitative RT-PCR

RNA was isolated with TRI Reagent® (Sigma-Aldrich) and transcribed to cDNA as previously described.[24] mRNA levels were normalized to the established housekeeping gene human β_2-microglobulin (B2M),[25] and results depicted as $\log_2$

fold increase over healthy control skin. Primers and probes for TaqMan qRT-PCR assays (Supplementary Table S1) were used from TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA).

Skin samples and histopathologic evaluation

Formalin-fixed skin specimens were embedded in paraffin, processed, stained with hematoxylin and eosin and analyzed by light microscopy. A board-certified dermatopathologist analyzed all samples in a blinded fashion. Assessment of histopathologic features was performed in a semi-quantitative fashion (Table 2), and graded according to a previously described histopathologic psoriasis scoring system (HPSS).[26,27]

Multicolor Immunofluorescence stainings and semi-automated analysis

Multicolor immunofluorescence stainings were performed on frozen sections,[28,29] and analyzed using Strataquest V64 (TissueGnostics GmbH, Vienna, Austria) in a semi-automated fashion (TissueFAXS), as previously established.[28] Briefly, a first set of analysis layers was applied to automatically differentiate between epidermal, upper and lower dermal areas (based on cell density/DAPI staining and background of immunofluorescence channels). The detected regions were manually controlled, and, if necessary, corrected by adding/substracting areas. NK cells and T cell subsets were gated based on mean immunofluorescence intensity of the respective channels, and cut-offs were adapted according to isotype controls.

Statistical analysis

B2M normalized mRNA expression values were $\log_2$-transformed prior to analysis. All analyses were carried out in R (www.R-project.org) and its available packages. Differences in expression values (in $\log_2$-scale) and cell counts were assessed using a linear mixed effect model. Once the model was fitted (using *lme* function), least square means were obtained, and group comparisons were assessed by a two-tailed t-test using contrasts (using functions *lsmean* and *contrast* in R package *lsmeans*). Condition (AD, Psoriasis, anti-TNF-induced lesion) was considered as fixed factor and Subject ID as random effect. With "lsmeans", Least Squared Means and Standard Error of the Mean for each Condition were estimated. The function "contrast" was used to estimate and contrast the differences between means (individual null hypothesis). The overall null hypothesis was tested by the "test" function, which executes a Fisher test. Statistical significance was assigned at $p \leq 0.05$. False discovery rates (FDR) were calculated using the Benjamini-Hochberg procedure (Supplementary Table S2).

RESULTS

Clinical appearance and histopathologic classification

We analysed nineteen biopsies from patients with new-onset anti-TNF-α related inflammatory skin eruptions (Table 1). Affected regions included the trunk, extremities, palms, soles, and the scalp. All scalp lesions were clinically accompanied by visible hair loss (alopecia). Hematoxylin/eosin (HE) stained specimens were assessed by a dermatopathologist for histological reaction patterns. Results were grouped according to an established histopathologic psoriasis severity score (HPSS)[26,27] and compared to findings suggestive of drug eruption (Table 2).

Seven lesions showed reaction patterns that were mainly consistent with psoriasis, reflected by a HPSS>13,[26,27] and were thus classified as "psoriasiform" (n=7). Seven samples yielded low HPSS scores, and displayed histopathologic features most consistent with spongiotic dermatitis (eczema). Three samples – all from the scalp – exhibited a mixed histopathologic reaction pattern with both psoriasiform and spongiotic features, consistent with an intermediate HPSS score. Two remaining biopsies yielded a low HPSS score and showed a dense lichenoid lymphocytic cell infiltrate at the dermo-epidermal junction, and were thus classified as lichenoid. Representative images of these 4 reaction patterns are given in Figure 1.

On a molecular level, psoriasiform and spongiotic/eczematous anti-TNF-induced skin lesions are distinct from both psoriasis and eczema

As anti-TNF-induced psoriasiform and spongiotic/eczematous skin lesions were histopathologically consistent with psoriasis and eczema (spongiotic dermatitis), respectively, we assessed these biopsies with an established molecular disease classifier.[30] This classifier consists of the two inflammatory mediators inducible nitric oxide synthase (NOS2 or iNOS) and CCL27, with high NOS2 and low CCL27 mRNA levels being characteristic of psoriasis, and low NOS2 and high CCL27 levels being characteristic of eczema.[30,31] Surprisingly, neither psoriasiform nor spongiotic/eczematous anti-TNF-induced lesions showed a molecular pattern characteristic of classic psoriasis or eczema (Figure 2A-D). Similarly, neither scalp lesions (Figure 2E-F) nor lichenoid lesions (Supplementary Figure S1A-B) showed expression levels in the range of psoriasis or eczema. From these data we conclude that anti-TNF-induced skin lesions are genuinely different from conventional

psoriasis or eczema, even if they show psoriasiform and spongiotic/eczematous histological features on conventional H&E staining.

Anti-TNF-induced skin lesions are characterized by strong IFN-γ expression across all subtypes

Next, we assessed 42 immune and barrier markers known to be involved in various inflammatory skin diseases[30,32] and depicted them in a heat map. Each group (psoriasiform, eczematous/spongiotic, scalp, and lichenoid lesions) was compared to conventional psoriasis and eczema (Figure 3A-C, Supplementary Figure S1C). In line with previous publications,[33] conventional psoriasis showed the highest levels of Th17-associated molecules (IL-17A, IL-17F, PI3/Elafin, CCL20, IL-19, S100A proteins), the antimicrobial peptide DEFB4, and the innate immune marker IL-1B, within each comparison (Figure 3A-C, Supplementary Figure S1C). Eczema showed highest levels of Th2-associated cytokines (IL-4, IL-13, CCL26, OX40L), the Th9 cytokine IL-9, and the thymic stromal lymphopoietin (TSLP) receptor, as previously described (Figure 3A-C, Supplementary Figure S1C).[15] Compared to psoriasis and eczema, all forms of anti-TNF-α-induced lesions showed higher increases in the Th1 lead cytokine interferon (IFN)-γ and in IFN-γ regulated molecules such as the IL-12 receptor subunit IL12RB2, and the CXCL9/CXCL10/CXCL11 chemokine receptor CXCR3 (Figure 3A-C, Supplementary Figure S1C), which, in turn, is classically found on Th1 cells. Across all anti-TNF-α lesions, we also found levels of the innate immune markers IFN-α1 and the antimicrobial peptide lipocalin (LCN)-2, both involved in Th1 priming,[34,35] to be higher than in psoriasis and eczema, but with lower levels in scalp samples than in psoriasiform or eczematous/spongiotic lesions (Figure 3A-C, Supplementary Figure S1C).

The strong IFN-γ signature in anti-TNF-α lesions is consistent with increased numbers of Th1, but not Tc1 or NK cells

To identify the potential cellular source(s) of the strong IFN-γ signature in anti-TNF-α lesions, we quantified cells that classically produce this cytokine, namely NK cells, type 1 T helper cells (Th1), and type 1 cytotoxic T cells (Tc1),[36] using a semi-automated histology analysis of multicolour immunofluorescence staining.[28] In both psoriasiform and spongiotic/eczematous lesions we found only very low numbers of NK cells, even lower than in healthy control skin (Figure 4A). By contrast, we identified a prominent infiltrate of T cells in anti-TNF-α lesions (Figure 4B). Among these, only Th1 cells (Figure 4C), but not Tc1 cells (Figure 4D) were significantly elevated compared to healthy control tissues. Scalp and lichenoid lesions were not assessed due to their small sample size.

Distinct cytokine pattern between psoriasiform, eczematous and scalp lesions

Apart from Th1 activation that was consistently strong in all histologic subtypes, anti-TNF-α-induced skin lesions showed some changes specific to the respective histologic subtype (Figure 5). Lichenoid eruptions were not assessed due to their limited sample size. Psoriasiform lesions showed normal levels of the skin barrier protein filaggrin (FLG), as opposed to decreases in eczematous and scalp lesions (Figure 5A). While both psoriasiform and eczematous lesions showed increases in the pro-inflammatory mediators IL-36A, IL-36G, IL-19, and IL-20, as well as the IFN-α marker Mx1, scalp lesions lacked upregulation of these mediators (Figure 5B-F). In contrast, only eczematous lesions showed upregulation of Th2-associated mediators such as IL-13, IL-5, and CCL26 (Figure 5G-I), and showed highest levels of the Th22-cytokine IL-22 not only compared to other anti-TNF-α lesions (Figure 5J), but

also compared to conventional psoriasis and eczema (Figure 3A-C, Supplementary Figure S1C).

DISCUSSION

By characterizing anti-TNF-induced inflammatory skin eruptions, we could stratify them into four histopathologic reaction patterns, with highest numbers of psoriasiform and eczematous lesions. However, we found that these eruptions are distinct from conventional psoriasis and eczema using a molecular disease classifier.[30,37] Also, biopsies from so-called inflammatory or psoriasiform alopecia of the scalp,[12] that showed histopathologic characteristics of both psoriasis and spongiotic dermatitis, did not meet molecular criteria of either disease. While the original classifier used a ratio built of NOS2 and CCL27, we decided to depict individual cohorts (fold change over healthy control), in line with the other markers investigated.

Despite their different histological patterns, all anti-TNF-α-induced lesions were unified by high IFN-γ responses that were higher than in conventional eczema or psoriasis. This immune axis was also strongly activated in 2 lichenoid samples. While the small sample size precluded their further characterization, they at least served as positive control for IFN-γ-dependent inflammation.[38]

The fact that among potential IFN-γ producing cell types, Th1 cells, but not Tc1 or NK cells, were most abundant, suggests the Th1 adaptive immune axis as a likely contributor to skin inflammation in these patients. Mechanistically, the strong Th1 response might be fueled by increases in IFN-α, a type I IFN, consistent with published reports.[3] TNF-α is thought to be an inhibitor of pDC development and IFN-α production.[18] Consequently, TNF-blockade might disturb a pre-existing cytokine

balance between TNF-α and IFN-α in affected skin, favoring pDC derived IFN-α secretion.[18] This notion is supported by previous reports showing increased staining intensities of Mx1 (alias MxA) protein in psoriasiform anti-TNF-α lesions,[3,17] a surrogate marker for type I interferon activity.[39] Conversely, it has been shown that the administration of IFN-α can induce psoriatic skin lesions,[20] and the application of imiquimod cream, a potent inducer of IFN-α, can exacerbate psoriasis.[40] IFN-α, in turn, can facilitate skin homing of T cells by the induction of CXCR3,[41] a receptor typically found on Th1 cells, which was also increased in all our samples, in line with previous reports in psoriasiform lesions.[3] In the peripheral blood, it has been shown that TNF-blockers increase expression of CXCR3 on circulating T cells in patients with rheumatoid arthritis, possibly promoting skin homing of these cells.[42] Furthermore, increases in IL12RB2, a receptor subunit of the pivotal Th1-polarizing cytokine IL-12 might be another component of an amplification loop of this type 1 immune response fueling skin inflammation during TNF-blockade.

While the role of IFN-α, especially in autoimmune diseases, has traditionally been linked to Th1 responses,[43] it has now also been associated with priming and expansion of Th17 cells.[44] Albeit most strongly increased in psoriasis, some Th17-associated mediators (IL-17A, S100A8, S100A9, S100A12, PI3/Elafin) were also upregulated in all anti-TNF-α-induced lesions, suggesting a contribution of the Th17 immune axis to the formation of these lesions. Importantly, a rare coding variant of the *IL23R* gene, which has previously been associated with increases in Th17 cytokine production,[45] has been described in patients with severe psoriasiform and/or anti-TNF-induced alopecia.[4]

It is well established that antimicrobial peptides such as human beta-defensin 2 (encoded by the DEFB4 gene) or cathelicidin antimicrobial peptide (CAMP)/LL37 are upregulated in psoriasis as compared to atopic eczema,[46] and they are thought to be critically involved in the pathogenesis of psoriasis via triggering autoinflammatory immune responses in dendritic cells.[21] In psoriasiform and spongiotic/eczematous anti-TNF-α lesions, CAMP/LL37 was upregulated at even higher levels than in psoriasis, mandating further investigation as to their pro-inflammatory contribution in these lesions.

Despite this consistent upregulation of Th1-associated mediators across all subsets of anti-TNF-induced lesions, we also found several important differences between them. In scalp lesions, the IFN-α/Mx1 component was missing. So far, "psoriasiform" alopecia has been recognized as part of a spectrum of anti-TNF-α-induced psoriasiform lesions, i.e. psoriasis of the scalp with patchy hair loss.[4,47] However, scalp lesions did not only lack IFN-α responses, they also showed low IL-19 and IL-20 levels, two cytokines classically upregulated in psoriasis,[33] that were also present in our psoriasiform and eczematous lesions. Moreover, scalp lesions lacked IL-36A and IL-36G, two IL-1-family cytokines classically upregulated in psoriasis.[48,49] It has been suggested that in anti-TNF-α-induced psoriasiform skin lesions, IL-36G sustains a proinflammatory self-amplifying loop with IL-17C,[23] and that T cell stimulation with IL-36 receptor ligands results in the induction of IL-17 and IFN-γ responses.[50,51] While IL-36A and IL-36G might have a pro-inflammatory role in psoriasiform and eczematous lesions outside the scalp, they are likely less relevant to anti-TNF-induced scalp lesions. Overall, and despite a small sample size, our data consistently demonstrate clear differences distinguishing anti-TNF-α-induced scalp

lesions not only from spongiotic/eczematous lesions, but also from psoriasiform lesions in body regions other than the scalp. We are also confident that the lower abundance of these cytokines in scalp biopsies was not due to the selection of only mildly inflamed lesions, as the same samples showed strong increases in S100A proteins, IL-17A, or PI3/Elafin, that were present at even higher levels than in e.g. eczema samples.

Lesions with a spongiotic/eczematous histopathologic reaction pattern differed from psoriasiform and from scalp lesions by increases in Th2-associated cytokines (IL-13/CCL26/IL-5), potentially explaining the eczematous histopathologic features of this anti-TNF-α subgroup, as these mediators are implicated as key pathogenic mediators in eczema.[52] Spongiotic/eczematous anti-TNF-α lesions also showed the highest levels of IL-22 among all groups, including psoriasis and eczema. This Th17/Th22 cytokine has been demonstrated to promote epidermal hyperplasia and to inhibit epidermal differentiation,[53] which makes IL-22 a potential trigger of the pathogenic disease phenotype in spongiotic (eczematous) anti-TNF-α lesions. Psoriasiform lesions, in turn, lacked downregulation of filaggrin (FLG), which is considered an important contributor to skin barrier, and has been implicated as being central to the development of eczema.[54]

Our patients, who mostly suffered from IBD, all developed skin lesions for the first time during TNF-α-blockade, limiting the results of this study to this specific patient group. Patients with pre-existing eczema and psoriasis can also flare under anti-TNF-α treatment,[55-58] but it remains to be determined whether their immune profile is similar to new-onset lesions. Another limitation of this pilot study is the small sample size, and results need to be confirmed in larger patient cohorts.

In sum, we demonstrate that new-onset psoriasiform, eczematous and scalp anti-TNF-induced skin lesions are distinct immunological entities with characteristic Th1/IFN-γ/IFN-α-associated inflammation and major differences from both conventional psoriasis and eczema.

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FIGURE LEGENDS

Figure 1. Anti-TNF-induced skin lesions. Representative hematoxylin and eosin stainings for anti-TNF-induced inflammatory skin lesions showing psoriasiform changes (A), spongiotic changes consistent with eczema (B), mixed psoriasiform and spongiotic changes found in scalp lesions that showed clinical signs of alopecia (C), and lichenoid changes (D).

Figure 2. Anti-TNF-induced skin lesions are different from psoriasis and eczema. mRNA levels of NOS2 and CCL27 are shown across histopathologic groups of anti-TNF-induced skin lesions. Psoriasiform (A), eczematous (B), and scalp lesions (C) are depicted as $\log_2$ fold change relative to healthy control skin, and compared to levels found in classic psoriasis and eczema (spongiotic dermatitis); data are normalized to β_2-microglobulin expression; 95%CI boxplot and LSmean±SEM; *P≤0.05, **P≤0.01, ***P≤0.001.

Figure 3. RT-PCR heat maps of mRNA expression levels. mRNA levels were normalized to β_2-microglobulin (B2M), and depicted as color-coded mean $\log_2$ fold changes (FCH) over healthy control skin; Red color denotes increases, and blue color denotes decreases relative to healthy control samples. Heat maps were assembled using non-hierarchical clustering. Samples of conventional psoriasis (plaque-type psoriasis, n=6) and eczema (atopic dermatitis, n=9) were used as comparators for each group of anti-TNF-induced inflammatory skin lesions, which

were stratified for histological criteria of psoriasiform (n=7) (A), spongiotic (n=7) (B), and scalp lesions (n=3) (C). Large frames denote clusters of maximal expression levels in psoriasis (blue), eczema (pink), and anti-TNF-induced lesions (black), respectively. Grey solid boxes mark molecules that were maximally upregulated in all groups of anti-TNF-induced lesions.

Figure 4. Quantification of interferon gamma (IFN-γ) producing immune cells. We assessed numbers of leukocytes in healthy controls (n=10), psoriasis (n=15), eczema (n=13), and anti-TNF-induced skin lesions (psoriasiform n=5, eczematous n=5), using markers for NK cells (A) and T cells (B). T cells were further divided into type 1 T helper cells (C) and type 1 cytotoxic T cells (D), as assessed by the type 1 transcription factor T-bet; 95%CI boxplot and LSmean±SEM of cell counts per mm epidermis; *P≤0.05, **P≤0.01, ***P≤0.001.

Figure 5. Differences between anti-TNF-induced psoriasiform, spongiotic/eczematous, and scalp lesions. Selected inflammatory and skin barrier molecules are shown for psoriasiform, spongiotic/ezematous, and scalp lesions induced by anti-TNF-α treatment; 95%CI boxplot and LSmean±SEM of $\log_2$ fold change in mRNA/B2M relative to healthy control skin; *P≤0.05, **P≤0.01, ***P≤0.001.

SUPPORTING INFORMATION

Supplementary Figure S1. Lichenoid anti-TNF-α lesions in comparison to psoriasis and eczema. mRNA levels of NOS2 (A) and CCL27 (B) in lichenoid eruptions shown as log_2 fold change relative to healthy control skin, compared to classic psoriasis and eczema; normalized to β_2-microglobulin expression; 95%CI boxplot and LSmean±SEM; *P≤0.05, **P≤0.01, ***P≤0.001. RT-PCR heat map (C) of mRNA expression levels normalized to β_2-microglobulin, depicted as mean log_2 fold changes (FCH) over healthy control skin, using non-hierarchical clustering. Red colour denotes increases, and blue colour denotes decreases relative to healthy control samples. Samples of conventional psoriasis (plaque-type psoriasis, n=6) and eczema (atopic dermatitis, n=9) were used as comparators for anti-TNF-induced lichenoid skin lesions. Large blue, pink and black frames denote clusters of maximal expression levels in psoriasis, eczema, and anti-TNF-induced lesions, respectively. Grey solid boxes mark molecules that were maximally upregulated in anti-TNF-induced lesions across all histological groups of anti-TNF-induced lesions.

TABLE 1. Baseline characteristics of study patients and histopathologic reaction pattern of anti-TNF-induced skin lesions.

Sample #	Diagnosis	Ongoing TNF-blocker treatment	Duration of anti-TNF therapy at time of biopsy (months)	Duration of skin lesions (months)	ANAs	Biopsy Location	Overall histopathologic pattern in biopsy
1	Ulcerative colitis	Adalimumab	59	9	negative	Lower Leg	Lichenoid
2	Crohn's Disease	Adalimumab	48	6	negative	Forearm	Psoriasiform
3	Crohn's Disease	Adalimumab	33	2	negative	Lower Leg	Psoriasiform
4	Crohn's Disease	Adalimumab	7	3	negative	Palm	Psoriasiform
5	Crohn's Disease	Adalimumab	35	1	negative	Palm	Spongiotic
6	Crohn's Disease	Adalimumab	8	2	negative	Palm	Psoriasiform
7	Crohn's Disease	Adalimumab	7	2	negative	Scalp	Psoriasiform-spongiotic
8	Crohn's Disease	Adalimumab	36	1	negative	Scalp	Spongiotic-psoriasiform
9	Crohn's Disease	Adalimumab	9	1.5	negative	Sole	Spongiotic
10	Crohn's Disease	Adalimumab	24	0.5	negative	Thigh	Lichenoid
11	Crohn's Disease	Adalimumab	12	2	negative	Trunk	Psoriasiform
12	Crohn's Disease	Adalimumab	11	8	negative	Trunk	Spongiotic
13	Rheumatoid Arthritis	Adalimumab	105	24	negative	Elbow	Psoriasiform
14	Rheumatoid Arthritis	Adalimumab	24	4	negative	Scalp	Spongiotic-psoriasiform
15	Crohn's Disease	Golimumab	11	6	negative	Trunk	Spongiotic
16	Ulcerative colitis	Infliximab	58	4	positive 1:320	Sole	Spongiotic
17	Crohn's Disease	Infliximab	24	3	negative	Hand	Psoriasiform
18	Crohn's Disease	Infliximab	44	1	negative	Trunk	Spongiotic
19	Crohn's Disease	Infliximab	36	12	positive 1:160	Trunk	Spongiotic

TABLE 2. Detailed histopathologic evaluation of anti-TNF-induced skin lesions.

Biopsy site		*Elbow*	*Palm*	*Lower leg*	*Hand*	*Palm*	*Trunk*	*Forearm*	*Scalp*	*Scalp*	*Scalp*	*Sole*	*Trunk*	*Palm*	*Sole*	*Trunk*	*Trunk*	*Trunk*	*Thigh*	*Lower leg*
Main categories (each 0 -12)	***Subcategories (each 0 - 3)***																			
Epidermal	Staggered parakeratosis	3	1	1	1	1	2	1	1	2	0	1	0	1	0	1	0	0	0	0
	Epidermal hyperplasia	3	3	2	2	1	2	2	1	2	3	2	0	2	1	1	1	1	3	1
	Hypogranulosis	2	2	2	1	0	1	1	0	1	0	1	2	1	0	1	0	0	1	0
	Mitosis	2	1	2	1	2	0	1	1	0	1	0	0	1	0	0	0	0	0	0
Vascular	Dilated vessels	2	2	1	2	2	1	1	2	1	1	1	2	1	2	1	1	1	1	1
	Elongated vessels	2	2	2	1	3	1	1	1	1	1	0	1	0	1	0	0	0	0	1
	Increased number	1	1	2	2	1	1	1	1	0	1	0	1	0	0	0	0	0	0	1
	Contorted vessels	1	1	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
Inflammatory cell infiltrate	Perivascular lymphocytes	1	3	2	1	1	1	2	2	1	1	2	1	1	2	1	1	1	3	1
	Neutrophils intraepidermal	0	0	0	1	0	1	2	2	0	0	1	0	0	1	0	0	0	0	0
	Neutrophils intracorneal	1	1	1	1	1	2	0	1	0	1	1	0	0	0	0	0	0	0	0
	Histiocytes in papillary dermis	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
HPSS summary (0 - 36)		***19***	***18***	***17***	***15***	***13***	***13***	***13***	***13***	***10***	***10***	***10***	***9***	***8***	***7***	***6***	***4***	***4***	***9***	***6***
	Continous hyper/parakeratosis	*0*	*0*	*1*	*0*	*3*	*0*	*1*	*0*	*1*	*1*	*0*	*0*	*0*	1	*0*	*1*	*1*	*0*	*0*
	Orthohyperkeratosis	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*0*	*1*	*0*	*0*	*1*	2	*0*	*0*	*0*	*1*	*2*
	Serumcrust	*1*	*2*	*0*	*0*	*1*	*0*	*0*	*0*	*0*	*1*	*0*	*0*	*0*	1	*1*	*3*	*3*	*0*	*0*
Other findings (n=13)	Hypergranulosis	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	1	1	1	1
	Dyskeratosis of epidermal KC	0	0	0	1	1	0	0	0	1	1	3	0	0	0	0	0	0	2	1
	Exocytosis of lymphocytes into the epidermis	0	1	1	0	1	0	0	1	1	1	2	1	1	2	2	3	3	2	1
	Spongiosis	1	1	1	1	1	1	0	1	1	1	3	1	1	3	2	3	3	1	0

	Vacuolar alteration of the basal membrane	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
	Edema of the papillary dermis	0	1	1	0	1	1	0	1	2	0	1	0	1	2	1	1	1	1	0
	Lichenoid lymphocytic infiltrate at the DEJ	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	3	3
	Eosinophils in the dermis	0	0	0	0	2	0	0	2	2	0	1	0	0	2	1	1	1	1	0
	Extravasated erythrocytes in the papillary dermis	0	0	1	0	0	0	0	1	0	0	1	0	1	0	1	1	1	0	0
	Plasma cells in the dermis	0	0	1	1	0	0	1	2	2	0	1	0	0	0	0	1	1	0	1
Total number of conflicting criteria (max = 13)		**2**	**4**	**6**	**3**	**8**	**2**	**3**	**8**	**9**	**6**	**7**	**2**	**5**	**8**	**6**	**9**	**9**	**9**	**6**
Overall pattern		**Psoriasiform**	**Psoriasiform**	**Psoriasiform**	**Psoriasiform**	**Psoriasiform**	**Psoriasiform**	**Psoriasiform**	**Spongiotic - psoriasiform, alopecia with foreign body reaction (granulomatous)**	**Psoriasiform - spongiotic**	**Spongiotic - psoriasiform, alopecia**	**Spongiotic**	**Spongiotic**	**Spongiotic**	**Spongiotic**	**Spongiotic**	**Spongiotic**	**Spongiotic**	**Lichenoid**	**Lichenoid**

Table 2. Histopathologic evaluation of anti-TNF-induced skin lesions. Semiquantitative analysis of major histopathologic patterns in anti-TNF-induced skin lesions, grouped for characteristics of psoriasis and drug eruptions, and summarizing the total number of criteria conflicting with a diagnosis of psoriasis, whenever applicable*; HPSS: histopathologic psoriasis severity score; n/a: not applicable; DEJ: Dermo-epidermal junction; KC: keratinocyte.*

Figure 1

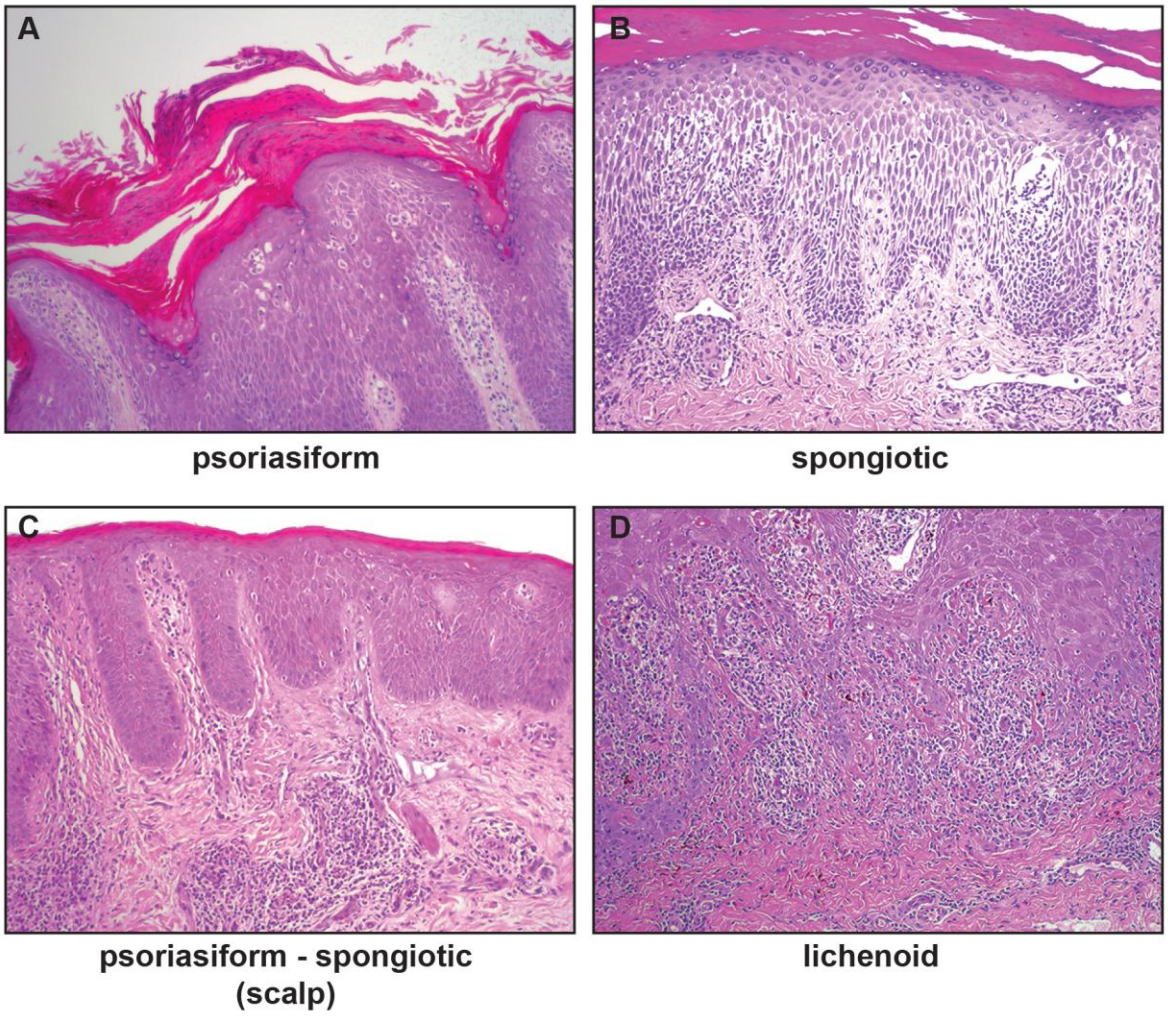
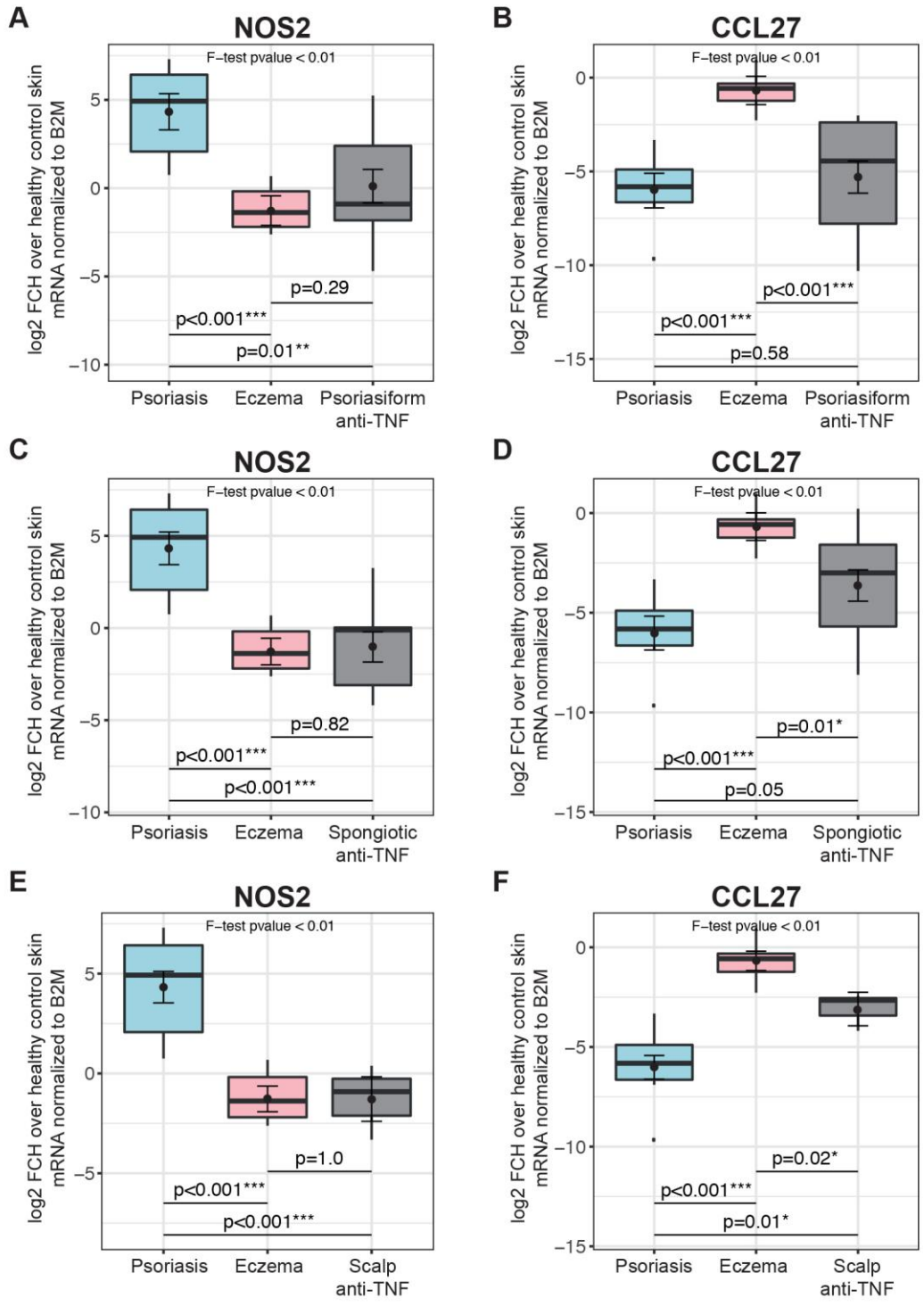


Figure 2



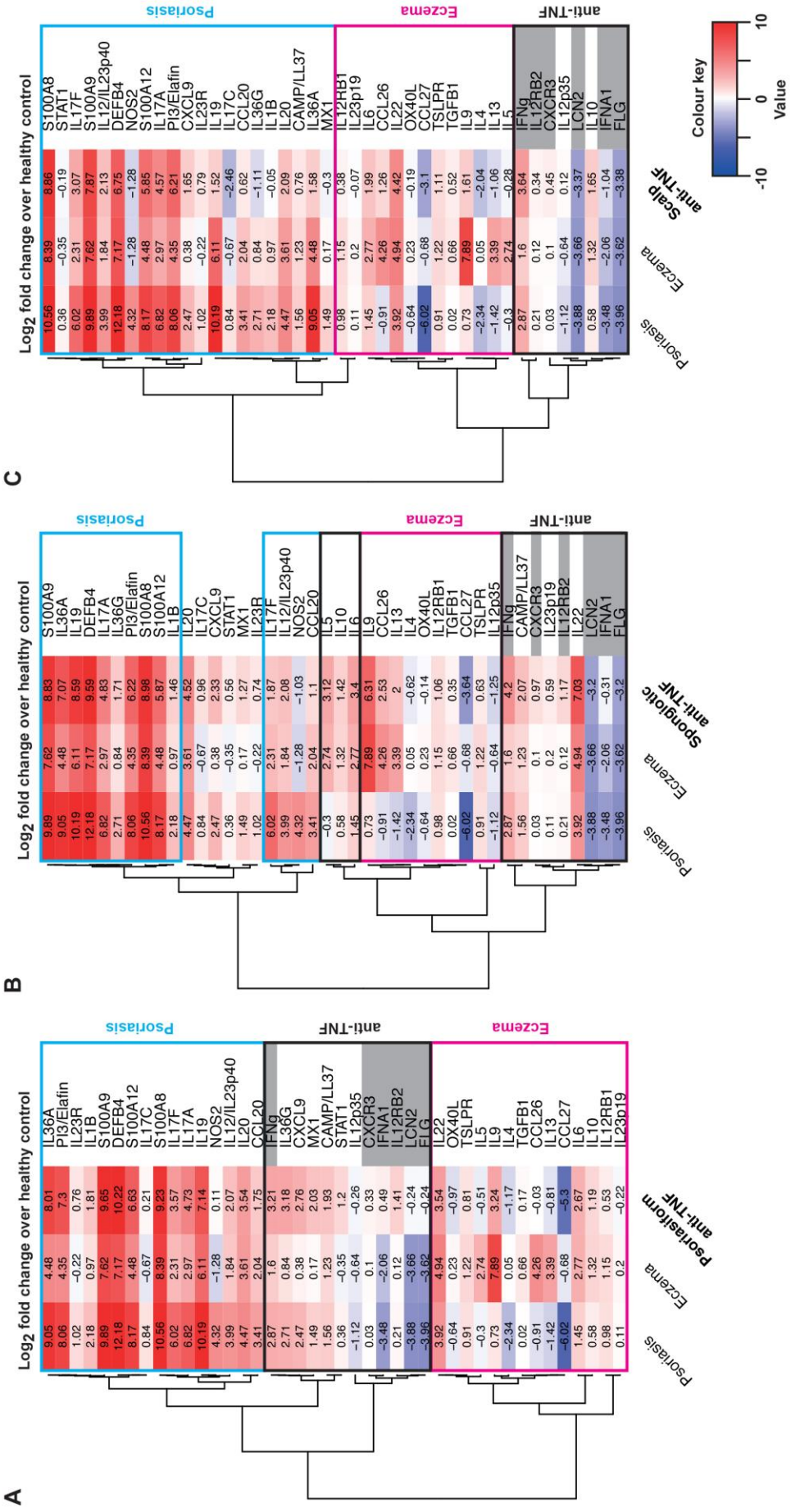


Figure 3

Figure 4

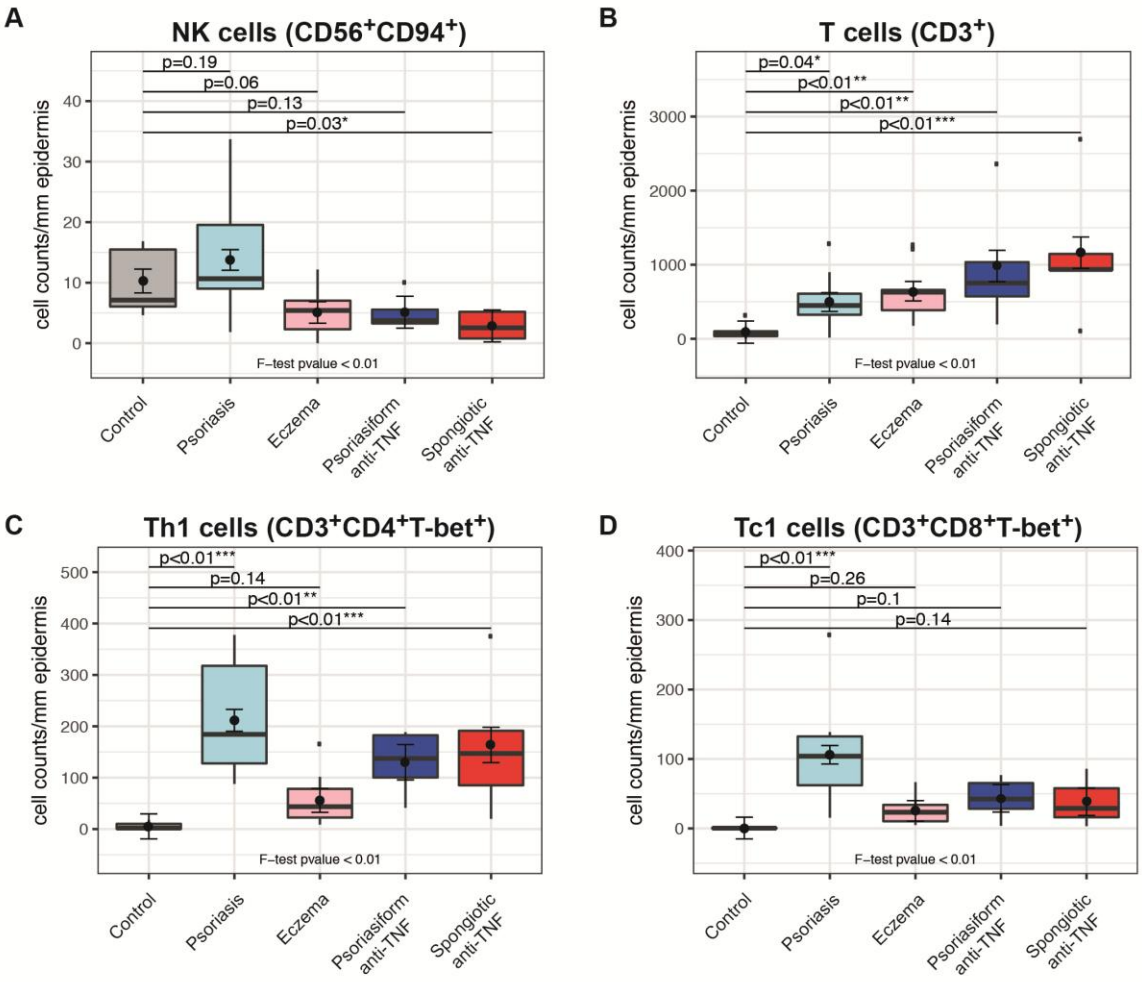


Figure 5